FINAL TECHNICAL REPORT

Project Title: Molecular Physiology of Nitrogen Allocation in Poplar / Molecular Determination of Carbon Sink Strength in Wood

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Problem Statement:
The forest products industry consumes large amounts of energy. Understanding how genetic variation in trees actually controls the characteristics of wood, the major raw material utilized by the industry, is an opportunity for energy savings. For companies that are vertically integrated (i.e., have both tree production and processing operations), energy savings can accrue for both production and processing. Tree production demands nitrogen fertilizers, the manufacture of which is highly energy intensive. Wood processing for paper product manufacturing requires digestion and bleaching, both of which are more efficient when the lignin content of wood is reduced. This project identified genes involved in utilization of nitrogen from fertilizer, and the coupling of nitrogen demand to lignin content, establishing a framework for reducing tree nitrogen demand per unit carbon gained. This creates opportunities for genetic manipulation of trees for greater energy efficiency.

Technical Approach:
The objectives of this research were to identify the early biochemical events that occur when poplars are fertilized, and to manipulate genes that are likely to play key roles in maintaining C:N balance within the tree. We first defined the response of trees to available nitrogen in short-term time course experiments. We then defined the spatial

and temporal sequence of events leading from nitrogen uptake to nitrogen utilization, thence to carbon gain (i.e., growth), and used that information to guide discovery of genes acting when nitrogen content is elevated, but growth has not yet occurred. Gene sequences indicated tight coupling of nitrogen and carbon-based metabolic pathways, and provided molecular indicators of nitrogen response, or a "nitrogen response roadmap" (NRR). The inferred function of the regulated genes were useful for monitoring the shifts in poplar gene expression that accompany increased N availability, and the downstream effects of N availability on carbon allocation and carbon partitioning. We used the NRR to identify processes that couple nitrogen availability and lignin content in stems, and directly manipulated the expression of candidate genes for carbon partitioning.

Because the project was carried out in two phases with distinct but complementary goals, the objectives are numbered by phase (I. Molecular Physiology of Nitrogen Allocation in Poplar, or II. Molecular Determinants of Carbon Sink Strength in Wood) and specific objective within each phase (1-3).

Objectives:

I.1.) To identify genes that are regulated in roots, stems, leaves and the shoot apex of poplar trees between the time N is applied to roots and the time increased growth is observed in the apex.

I.2.) To produce transgenic trees that have altered protein levels showns previously to be involved in nitrogen allocation. (Transgenic plants were made, but protein levels were not significantly altered. Consequently this objective was re-worded to more accurately reflect the scientific objective, which was: To experimentally perturb poplar tree nitrogen allocation *in vivo*).

I.3.) To analyze the transgenic lines for altered growth characteristics, N and carbon transport/storage compounds, photosynthesis and gene regulation under conditions of low and elevated N.

II.1.) To develop a "wood growth roadmap" of genetic, molecular and biochemical processes controlling wood formation and wood properties.

II.2.) To determine how light and fertilization affect the expression of the wood growth roadmap that controls wood formation and wood properties.

II.3.) To produce and analyze transgenic *Populus* trees with increased capacity to import C resources into wood.

Project Schedule:
December 1997, Project initiation. December 2003, Original expected completion date negotiated at project award. December 2004, Revised completion date per contract modifications. All milestones were met.

Table 1. Dates of Major Milestones and Go/No-Go Decision Points.

	Major Milestone	Accomplished?
12/98	Gene constructs for transgenic lines (anti-rbcS)	yes
12/99	Clone 40 N response "sentinel" genes	yes
12/99	Transgenic lines made by industry cooperator	yes
12/00	Establish field trials in Puyallup, WA	no (No-Go)[1]
12/01	Gene constructs for transgenic lines (IVR, SUS)	yes
12/02	Complete "nitrogen response roadmap" in all tissues	yes
12/02	Transgenic lines made by industry cooperator	yes
12/04	Completion of transgenic tree phenotyping	yes (IVR) (SUS No-Go)[2]

[1]Explanation of field trial No-Go: Transgenic lines were made, screened, confirmed to be transgenic, but the nitrogen partitioning phenotype was expressed only at the mRNA and not at the protein and whole-plant levels. With no discernible physiological phenotype, we shifted resources toward additional "sentinel" gene discovery in order to make the nitrogen response roadmap more comprehensive.

[2]Explanation of SUS No-Go: SUS transgenic trees were made, screened, confirmed to be transgenic, but the SUS transgenic lines deteriorated in culture. We shifted resources toward characterization of IVR lines, and manipulative physiology experiments on stems in order to create clear linkages between expression of our cloned "sentinel" genes, nitrogen availability, and measurable shifts in lignin content in developing wood.

Accomplishment of Objectives:

I.1.) To identify genes that are regulated in roots, stems, leaves and the shoot apex of poplar trees between the time N is applied to roots and the time increased growth is observed in the apex.

We first developed methods to deliver controlled levels of N to poplar trees and monitor tissue N levels in concert with growth. This experimental system, newly developed by our lab through this project, rigorously controls the N levels delivered to plants and allows the dramatic plasticity of poplar response to N to be manipulated and quantified. The following photo, taken of representative plants from the low, medium and high N treatments after 28 days, illustrates the dramatic plasticity of poplar response to N.

Photograph showing poplar response to available N. Plants were grown to ~60 cm in height under adequate N conditions (1 mM ammonium nitrate twice weekly) and then provided with 0 mM ("limiting"), 2 mM ("adequate") or 50 mM ("luxuriant") levels of ammonium nitrate for 28 days. The dark green foliage, large leaves and sylleptic branching distinguish luxuriant plants from limiting plants.

The methods development and results of these experiments are reported in Cooke et al. (2003. Plant, Cell and Environment 26: 757-770). The results show that at 7 d after treatments commence there is an increase in N but growth has not yet been initiated. This result is highlighted in the following figure:

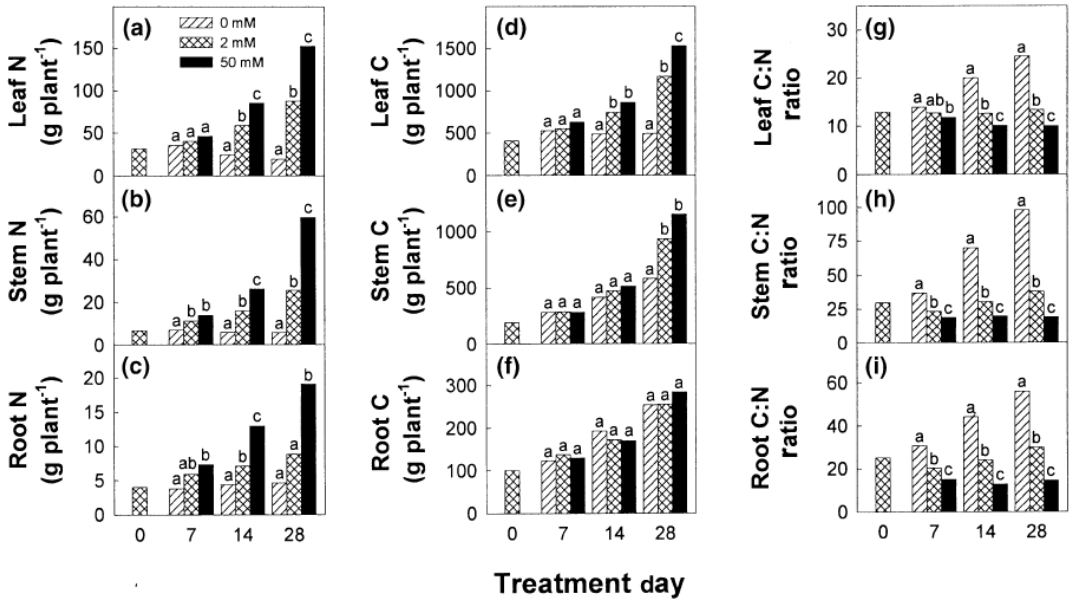


Figure 1. Increasing N availability is correlated with increased N and C allocation to leaves and stems, and a concomitant shift in the C : N ratio. Plants were treated with 0 mM (diagonally hatched bars) 2 mM (cross-hatched bars) or 50 mM (solid bars) NH_4NO_3 for up to 28 d. Total N in (a) leaves (b) stems and (c) roots. Total C in (d) leaves (e) stems and (f) roots. C : N ratio in (g) leaves (h) stems and (i) roots. Data are the means of three separate experiments, with at least two plants per treatment per day per experiment. ANOVA and multiple comparison of means were used to determine significant differences between 0, 2 and 50 mM NH_4NO_3 treatments for each tissue for each day ($\alpha = 0.05$). Means assigned the same letter within a grouping are not significantly different.

Using these plant treatment methods, ~100 genes were identified that were regulated in response to N but prior to growth (i.e., at 7 days after treatment). These results are reflected in the public release of all DNA sequence data to GenBank. A table containing a regulated subset of these genes is presented below:

Table 1. Nitrogen-responsive cDNA clones identified by differential display in *Populus trichocarpa* × *deltoides*

Clone	Length (bp)	Accession no	Related sequence	E value
cDNAs with putative function				
bsp	1153	CAA49669	*Populus* bark storage protein	–
win4	1112	AAA16342	*Populus* vegetative storage protein	–
pni288	934	AF330050	*Populus* win4 vegetative storage protein (AAA16342)	3×10^{-51}
pni69	695	BU791162	*Lycopersicon* ADP-glc pyrophosphorylase large subunit (U85497)	3×10^{-73}
pni88	271	BU791222	*Lycopersicon* osmotin-like protein (L76632)	3×10^{-4}
pni95	201	BU791224	*Arabidopsis* similar to protein phosphatase 2C (ABI1-like) (AAD46006)	2×10^{-19}
pni102	387	BU791128	*Medicago* histone H3 (U09460)	1×10^{-52}
pni107	537	BU791129	*Solanum* soluble starch synthase (P93568)	2×10^{-28}
pni122	835	BU791131	*Arabidopsis* similar to bHLH regulatory proteins (AL023094)	3×10^{-46}
pni134	430	BU791225	*Brassica* pollen coat protein similar to cold-induced kin1 (BAB10133)	7×10^{-7}
pni145	592	BU791172	*Arabidopsis* similar to chloroplast 31 kDa RNA-binding protein (CAB78028)	8×10^{-40}
pni150	1250	BU791137	*Arabidopsis* glutathione-S-transferase (U70672)	3×10^{-37}
pni159	705	BU791139	*Citrus* thiazole biosynthetic enzyme (Z82983)	2×10^{-84}
pni164	267	BU791176	*Nicotiana* kinesin-like calmodulin-binding protein	2×10^{-22}
pni183	731	BU791143	*Arabidopsis* similar to recA and LRR proteins (AAG51016)	3×10^{-29}
pni212	583	BU791147	*Glycine* SE60 sulfur-rich protein similar to proteinase inhibitor II (Z18359)	5×10^{-19}
pni220	505	BU791148	*Arabidopsis* ABC transporter-like protein (BAB10074)	2×10^{-39}
pni236	830	BU791150	*Arabidopsis* similar to protein disulfide isomerase (AAD46003)	4×10^{-35}
pni240	540	BU791152	*Lavatera* LTCO11 similar to GASA, gibberellin-regulated proteins (AF00784)	9×10^{-24}
pni263	771	BU791154	*Gossypium* sesquiterpene cyclase (U88318)	1×10^{-42}
pni275	259	BU791195	*Arabidopsis* similar to ser hydroxymethyltransferase (AAG40343)	5×10^{-10}
pni278	776	BU791156	*Arabidopsis* similar to vacuolar sorting receptor (AAF98196)	3×10^{-31}
pni282	492	BU791157	*Lycopersicon* histone H1 (AF253416)	5×10^{-6}
pni287	461	BU791158	*Malus* proline rich protein (T17107)	2×10^{-20}
pni289	409	BU925847	*Pyrus* Rubisco ss (D00572)	9×10^{-38}
po109	318	AY166668	*Populus* photosystem Q(B) protein (P36491)	3×10^{-56}
po164	424	AY166669	*Fragaria* hydroxyproline rich protein (AAD01800)	4×10^{-14}
pot171	536	AY161276	*Populus* Caffeoyl-CoA 3-O-methyltransferase (AJ224895)	3×10^{-75}
cDNAs with unknown function				
pni22	617	BU791149	No similarity	–
pni27	471	BU791155	*Populus* cambial region EST A047P47 U (AI163751)	3×10^{-4}
pni28	409	BU791204	*Arabidopsis* unknown protein (AAF27106)	2×10^{-20}
pni48	323	BU791211	No similarity	–
pni68	160	BU791218	No similarity	–
pni120	503	BU791130	*Arabidopsis* unknown protein (AAK59650)	2×10^{-26}
pni123	535	BU791165	No similarity	–
pni125	358	BU791166	*Populus* cambial region EST A010P08 U (AI161945)	1×10^{-130}
pni130	571	BU791133	No similarity	–
pni135	248	BU791169	No similarity	–
pni138	335	BU791170	*Populus* leaf EST CO74P73 U (BI072388)	5×10^{-58}
pni143	189	BU791171	No similarity	–
pni151	212	BU791173	*Populus* cambial region EST A031P41 U (AI163072)	1×10^{-5}
pni171–1	298	BU791178	*Populus* developing bud cDNA bd8–1 (BF299456)	1×10^{-83}
pni175	243	BU791181	*Arabidopsis* unknown protein (AAD21437)	1×10^{-4}
pni177	269	BU791182	*Arabidopsis* unknown protein (AAF21213)	3×10^{-22}
pni180	481	BU791141	*Arabidopsis* unknown protein (BAB11541)	2×10^{-19}
pni182	534	BU791141	*Populus* cambial region EST A028P59 U (AI63003)	1×10^{-56}
pni184	508	BU791144	*Arabidopsis* unknown protein (CAB86048)	5×10^{-23}
pni187	482	BU791145	*Populus* leaf EST C062P01 U (BI071672)	7×10^{-64}
pni218	402	BU925848	*Cicer* tissue specific protein (X97455)	6×10^{-6}
pni242	398	BU791190	*Arabidopsis* unknown protein (AAK59486)	6×10^{-17}
pni246	595	BU791153	*Arabidopsis* unknown protein (AAG52577)	4×10^{-49}
pni279	426	BU791199	*Arabidopsis* unknown protein (AAD32907)	3×10^{-29}
pni285	375	BU791201	No similarity	–
pni286	300	BU791203	*Populus* leaf EST CO74P73 U (BI072388)	4×10^{-52}

The length of the 3′ cloned partial cDNA is given in base pairs. Sequence similarities based on BLASTN (ESTs) or BLASTX (others) (NCBI). The GenBank accession number of the sequence demonstrating the highest similarity is indicated in parentheses.

We utilized membrane-based gene expression arrays to identify genes that were differentially regulated in leaves, stems, roots and the shoot apex. These results are presented in the figure below:

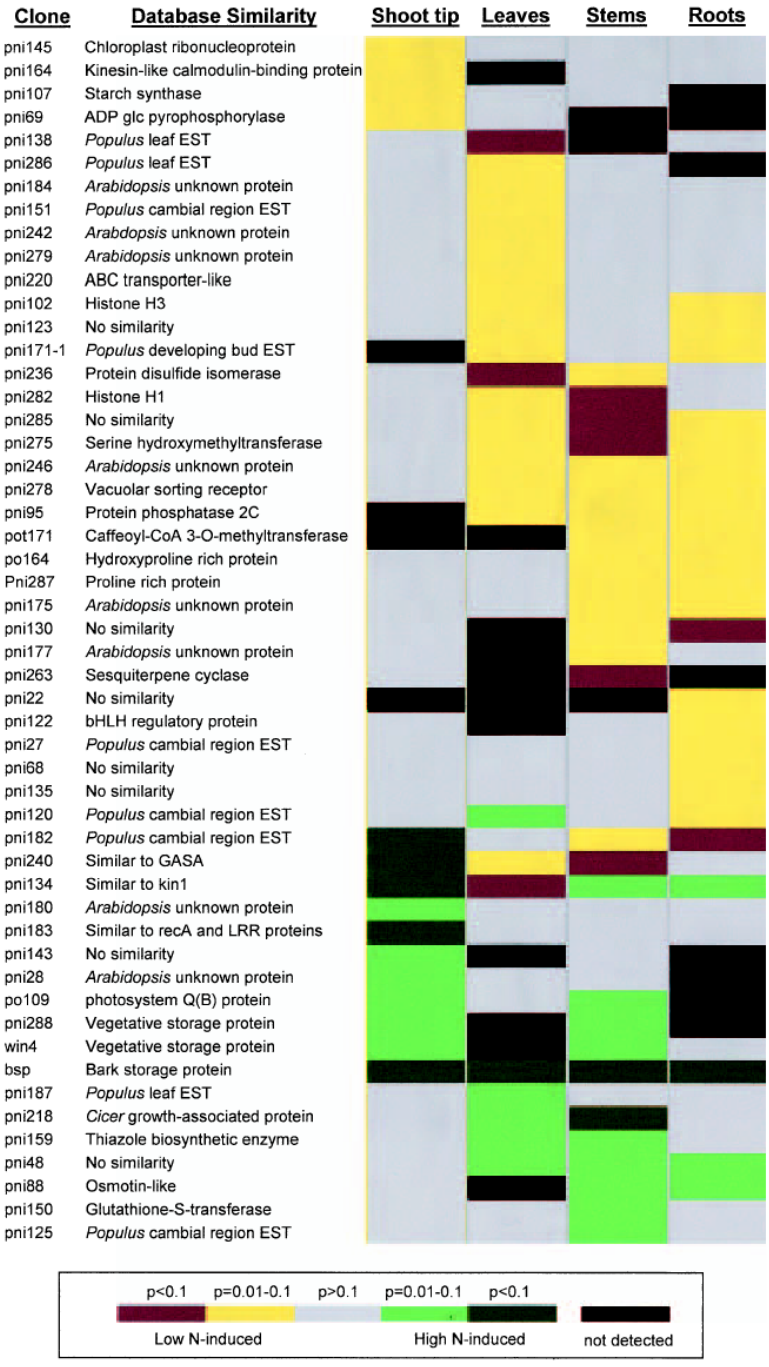
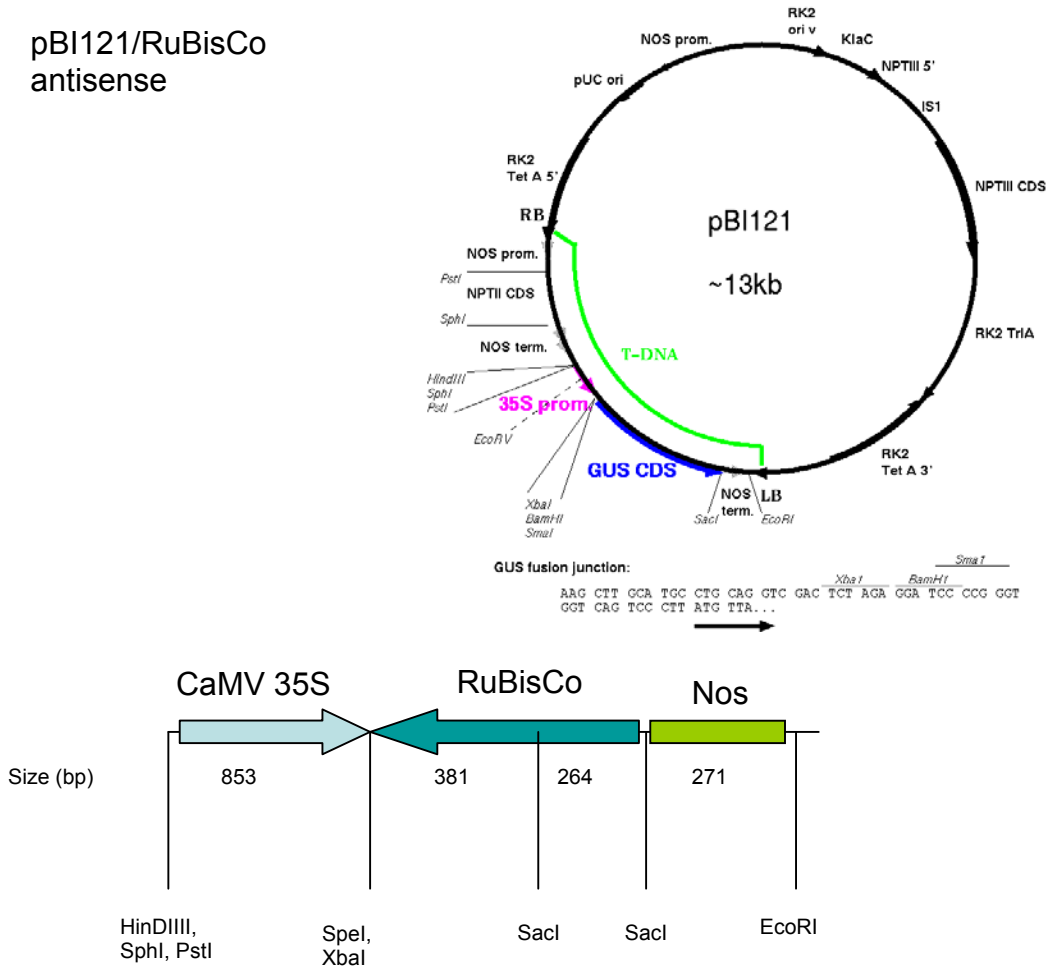


Figure 2. A nitrogen-response roadmap in poplar. Filters arrayed with cDNAs were probed with ^{32}P-labelled first-strand cDNA synthesized from 10 μg total RNA extracted from tissues of plants treated with 0 or 50 mM NH_4NO_3 for 16 d. *t*-tests were used to statistically compare scaled transcript abundance values in 0 versus 50 mM NH_4NO_3 for each tissue for each cDNA. The cDNAs showing significantly greater transcript abundance with 0 mM NH_4NO_3 treatment than with 50 mM NH_4NO_3 treatment were considered to be low-N induced, and are shown in yellow ($P = 0.01$–0.1) or red ($P < 0.01$). The cDNAs showing significantly greater transcript abundance in 50 mM NH_4NO_3 treatment than with 0 mM NH_4NO_3 treatment are considered to be high-N induced, and are shown in light green ($P = 0.01$–0.1) or dark green ($P < 0.01$). Light grey indicates no significant difference in transcript abundance was detected between treatments. Dark grey indicates that the mean transcript abundance in both 0 and 50 mM NH_4NO_3 treatments was below the mean + SD transcript abundance for a low-abundance reference cDNA. Individual cDNAs were replicated three times.

I.2.) To produce transgenic trees that have altered protein levels showns previously to be involved in nitrogen allocation.

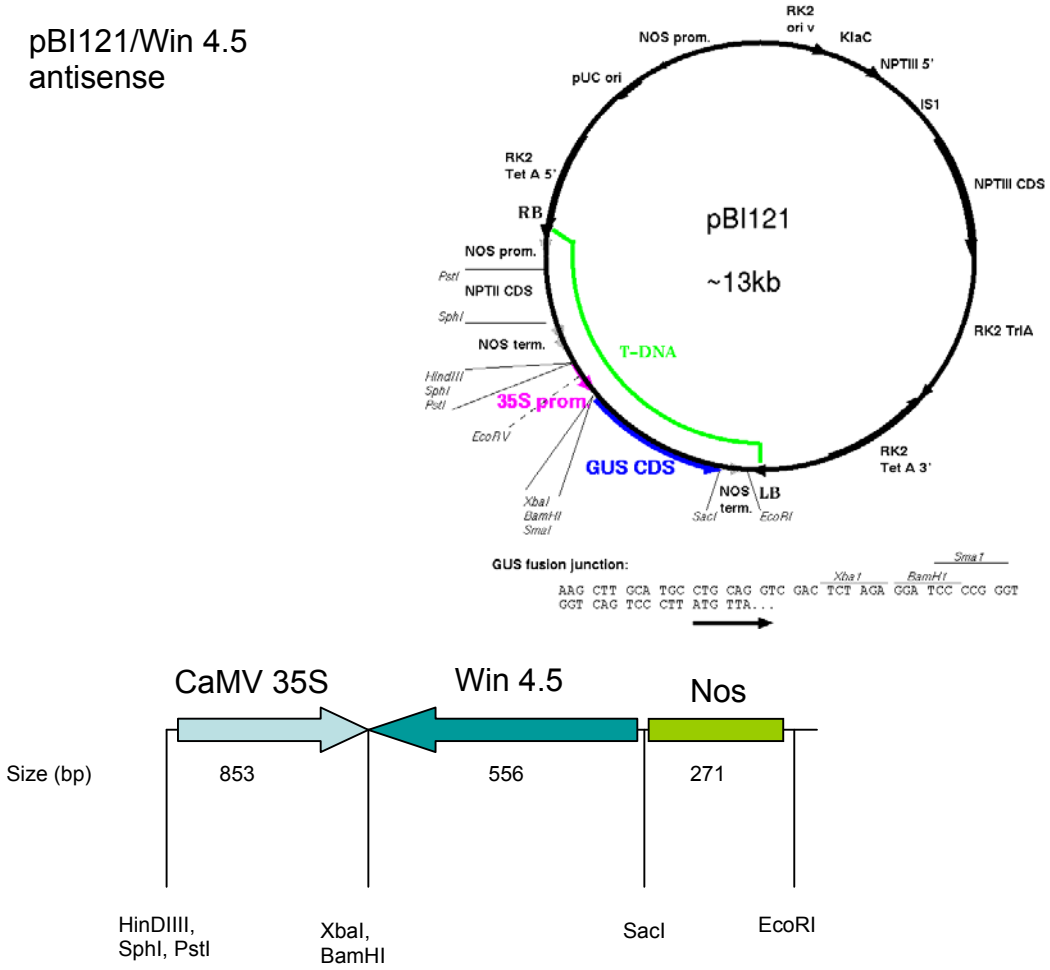
The target genes for manipulation were the small subunit of RuBisCO (antisense), the WIN4 Vegetative Storage Protein (antisense) and Nitrate Reductase (a constitutively active mutant form). A diagram of the RuBisCO antisense binary vector that we constructed in this project is shown below:

pBI121/RuBisCo antisense



Antisense rbcS construct cloned into the pBI121 binary vector backbone. The poplar cDNA was cloned by PCR from reverse-transcribed mRNA collected from young leaves, ligated into the vector, and verified by sequencing.

A diagram of the WIN4 antisense binary vector that we constructed in this project is shown below:

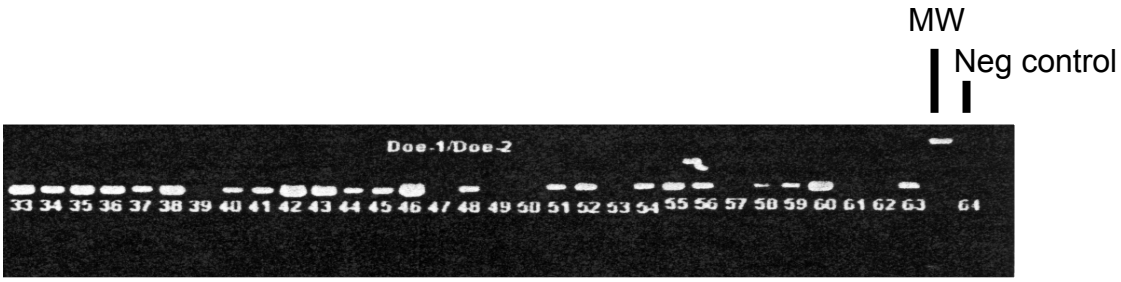


Antisense win4 construct cloned into the pBI121 binary vector backbone. The poplar cDNA was cloned by PCR from reverse-transcribed mRNA collected from young leaves, ligated into the vector, and verified by sequencing.

The mutant Nitrate Reductase construct was not used for several reasons including a shift in the interest of our intended collaborator away from this project, and a less-than-anticipated impact of the mutation on the whole-plant phenotype when the transgene was introduced into model plants.

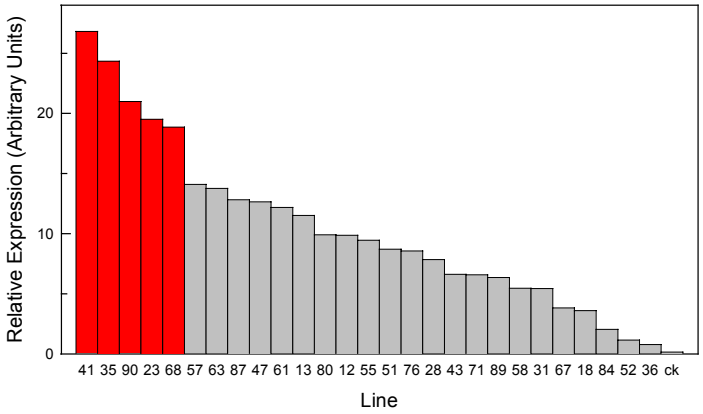
Introduction of the transgene into poplars was carried out by our collaborator at Union Camp. Due to circumstances beyond our control, at a critical time during the antibiotic selection of transgenic shoot materials the transgenic plant laboratory of Union Camp was moved to another state and the plant materials were prematurely placed on nonselective medium. After regeneration the plants entered dormancy. We spent a significant amount of time characterizing the plant materials by: 1) initiating cold treatments to meet the vernalization requirements of the plant lines, 2) transferring plants

to greenhouse conditions to stimulate growth, and 3) attempting to verify the presence and/or expression of the antisense transgenes in the newly propagated plant material. Unfortunately we could not verify transgene presence or expression in the lines. To meet the objectives of this project we sent the constructs to another collaborator, Lailiang Chiang, at Oregon State University. He verified the presence of the RuBisCO (rbcS) transgene in many, newly created, transgenic lines as illustrated in the following figure:



Transgenic line screening for antisense rbcS plant lines of poplar. PCR products were generated using binary vector-specific primers and run on an agarose gel prior to being photographed. MW, molecular weight size marker. Neg control, a sample lacking template DNA.

We verified expression of the transgene by examining steady-state transcript levels in various transgenic lines as illustrated in the following figure:

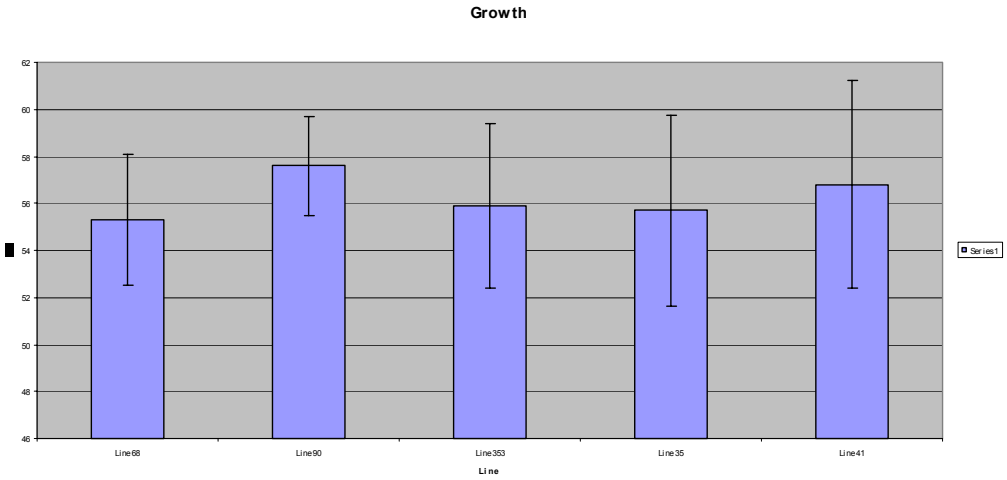


Transgenic line screening for expression of the antisense rbcS construct in poplar. RNA gel blots were probed with the transgene-specific NOS terminator and are ranked according to expression level from left to right. The lines shown in red were evaluated for reduction in steady-state rbcS transcript as well and were confirmed to have reduced levels of rbcS transcript compared to nontransgenic lines. CK, expression of NOS in a control line lacking a transgene.

Transgenic lines expressing an antisense version of win4 were not obtained. The reasons for this are not clear, but we speculate that the expression of an antisense win4 may alter nitrogen source-sink relationships among the cells in tissue culture during the shoot regeneration process. Armed with antisense rbcS plant lines, we proceeded to the next stages of the project.

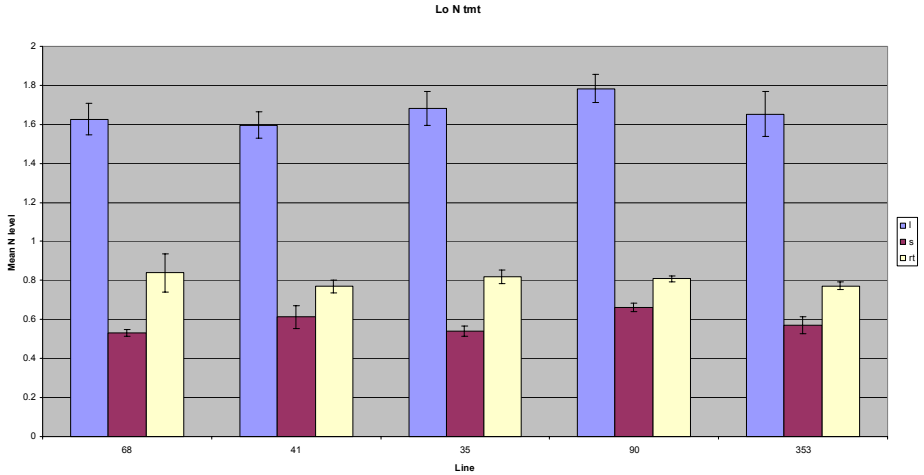
I.3.) To analyze the transgenic lines for altered growth characteristics, N and carbon transport/storage compounds, photosynthesis and gene regulation under conditions of low and elevated N.

The transgenic plant lines were assessed for altered growth characteristics by using the variable N treatment method that we had developed in the initial stages of the project. Under conditions of limiting, adequate or luxuriant N there were no growth differences between nontransgenic and transgenic plant lines as illustrated by the figure below:



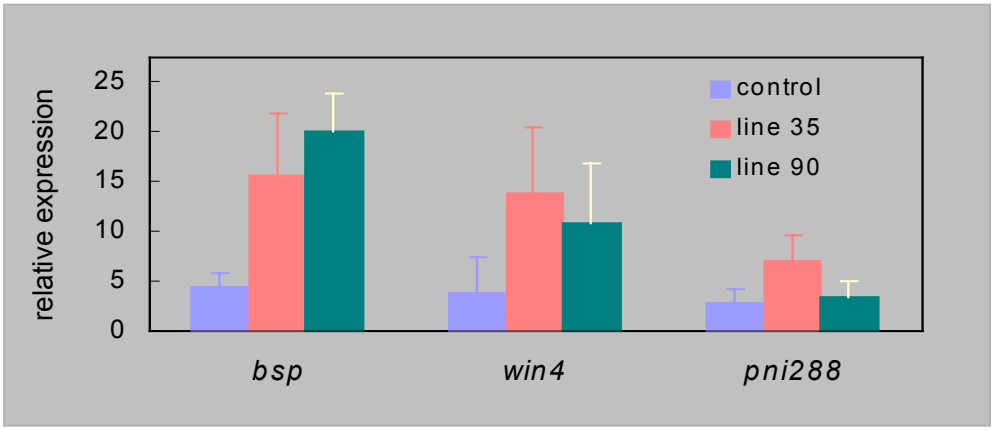
Growth does not differ significantly between transgenic rbcS antisense lines and nontransgenic controls. From left to right, the genotypes are transgenic line 68, transgenic line 90, nontransgenic control, transgenic line 35 and transgenic line 41. The y-axis begins at 46 cm and ends at 62 cm.

Nitrogen content and photosynthesis (Amax as well as ACI curves were determined) did not differ between transgenic and nontransgenic lines. Representative negative data for N content are shown below:



Nitrogen concentration does not differ between transgenic antisense rbcS plant lines and nontransgenic controls. N concentration values are shown for leaves (blue bars), stems (magenta bars) and roots (tan bars) from transgenic lines 68, 41, 35, 90 and nontransgenic controls presented left to right respectively. These plants were grown under low N but the same trends were seen for high N (data not shown).

Notably, a gene expression phenotype was observed for the antisense rbcS plant lines compared to the nontransgenic control. When the transgenic lines were grown under conditions of limiting N, transcript abundance for storage proteins BSP, WIN4 and PNI288 were elevated in the transgenic lines. This result is consistent with the role we have proposed for VSPs in nitrogen allocation within poplar trees (Lawrence et al. 2001. *Canadian Journal of Forest Research* 31: 1098-1103) and highlights the potential role of RuBisCO as a storage protein in plants.



Steady-state transcript abundance for the three major storage proteins in poplar trees, BSP, WIN4 and PNI288. RNA gel blots were hybridized with gene-specific probes and then quantified by phosphoimagery.

II.1.) To develop a "wood growth roadmap" of genetic, molecular and biochemical processes controlling wood formation and wood properties.

Defining the wood growth roadmap requires that we focus on the xylem, which is the tissue type that makes wood. This focus on xylem differs from the whole-plant perspective that we developed in the first phase of the project. Examples of genes that we identified in the first phase of the project that are expressed preferentially in xylem are shown in the figure below.

We placed these newly discovered genes into the context of a wood development roadmap by combining our data with existing literature. We authored a comprehensive book chapter that summarizes our findings and places individual genes within a context of wood growth. The book chapter is attached to this report.

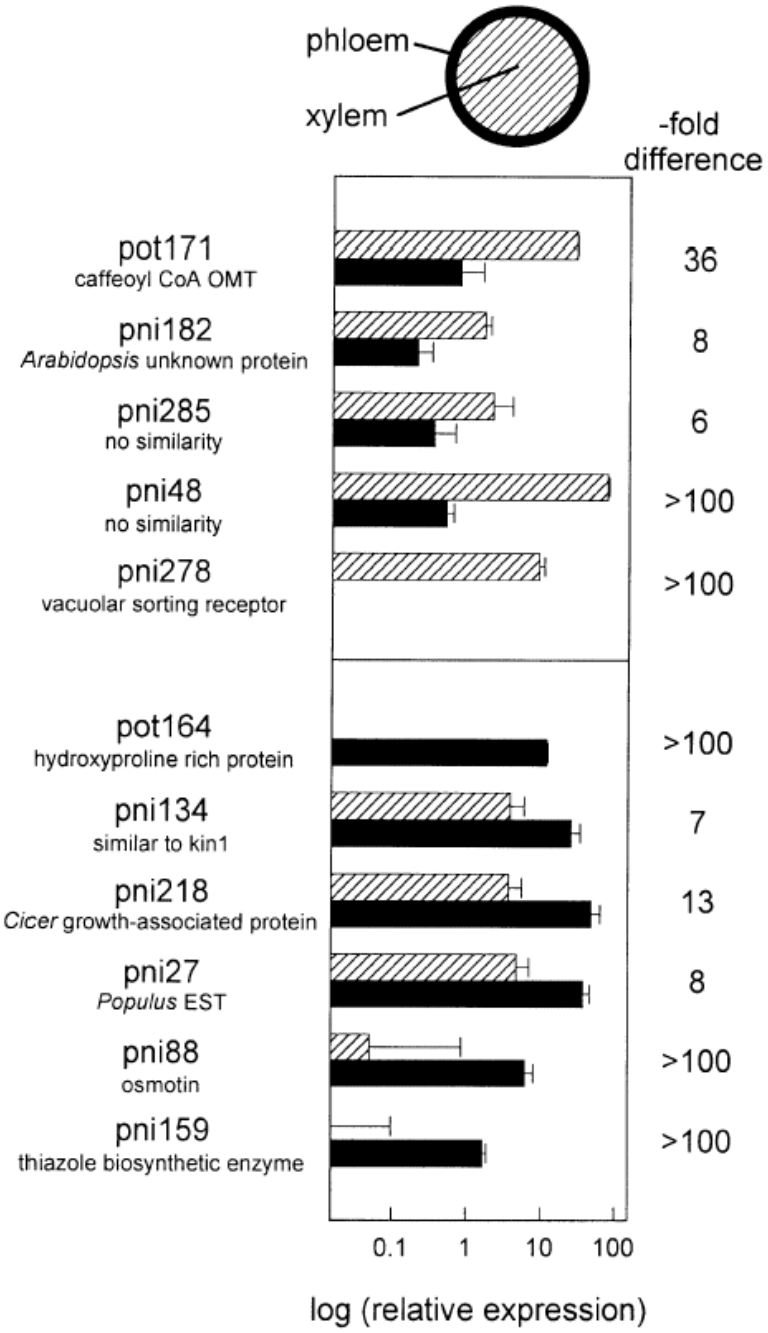
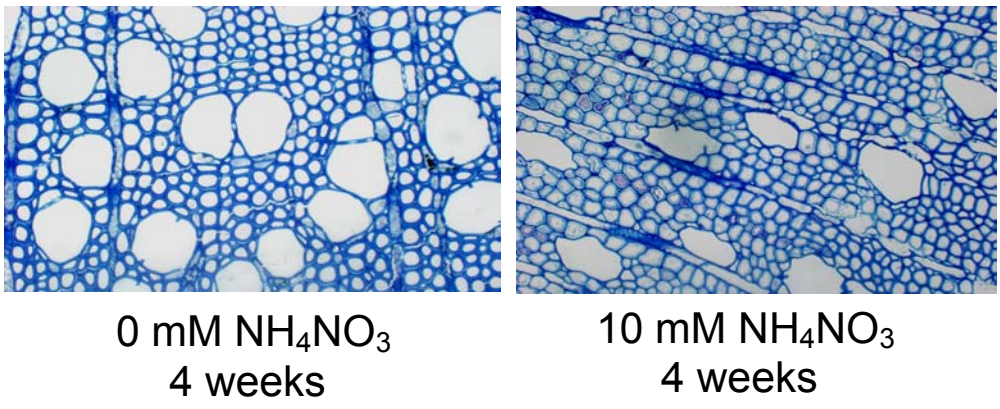


Figure 3. Several stem N-responsive cDNAs are preferentially expressed in xylem or phloem. Trees were treated for 3 weeks with 2 mM NH_4NO_3. Filters were probed with ^{32}P-labelled cDNA synthesized from 8 μg total RNA. Transcript abundance is expressed as the $\log_{10}$ values of $\bar{x} = 10(\Sigma x_i/s)/n$ (see Materials and methods). Individual cDNAs were replicated three times. Plotted values for xylem (cross-hatched bars) and phloem (solid bars) are means ± SD. Values to the right of each bar pair indicate the fold difference in expression of a cDNA between xylem and phloem.

II.2.) To determine how light and fertilization affect the expression of the wood growth roadmap that controls wood formation and wood properties.

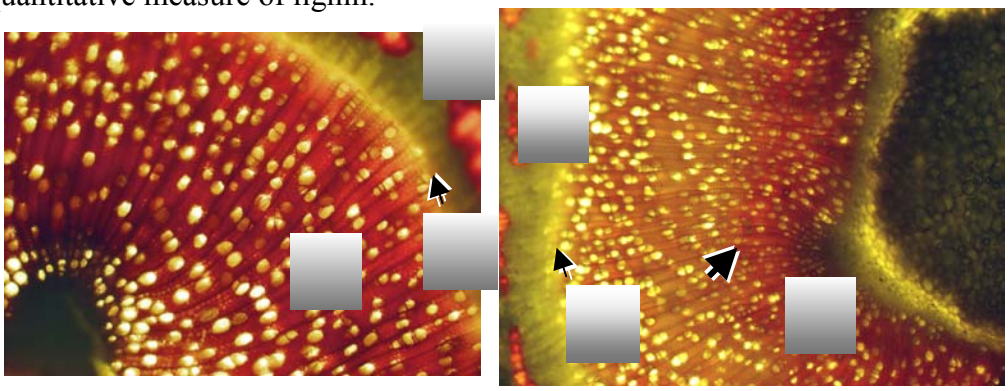
Our research on wood development gave us new insights and led us to modify this objective somewhat. We still felt as though the emphasis on fertilization was appropriate, however we opted to use phloem girdling instead of light (photoperiod) to alter wood quality. We reasoned that the effects of girdling were likely to be manifest in a shorter time frame than photoperiod. Furthermore, we discovered that girdling is a reproducible method to create a dramatic nitrogen gradient within a short distance in developing xylem. In this section our discoveries of the impact of nitrogen on wood structure and wood chemical properties will be highlighted.

The impact of fertilization on wood quality was rapid and dramatic, as the micrographs below reveal.



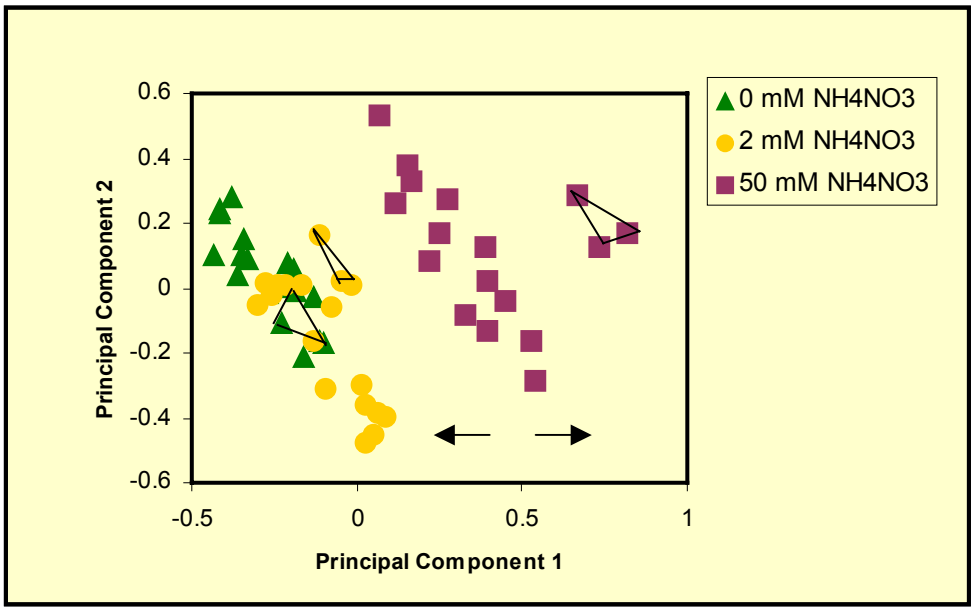
Nitrogen fertilization alters cellular structure and appears to affect cell wall properties. The upper panels are light micrographs of xylem after four weeks of treatment under limiting (left) and luxuriant (right) levels of nitrogen. The lower panels are transmission electron micrographs of the same tissues, and reveal enlarged cell walls.

The data on cellular structure suggested that the chemical properties of the cell walls may be altered by nitrogen availability. To test this idea, tissue sections were stained with phloroglucinol, a compound that stains cinnamylaldehyde residues and is thus a semiquantitative measure of lignin.



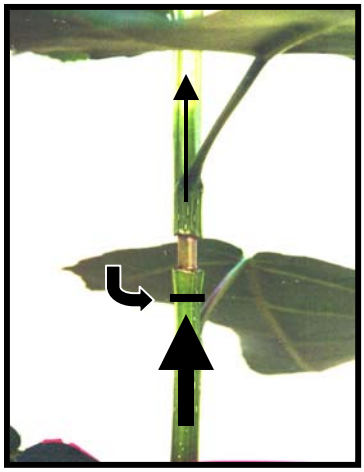
Phloroglucinol staining indicates a reduction in lignin concentration in xylem cells of trees grown under conditions of high nitrogen availability.

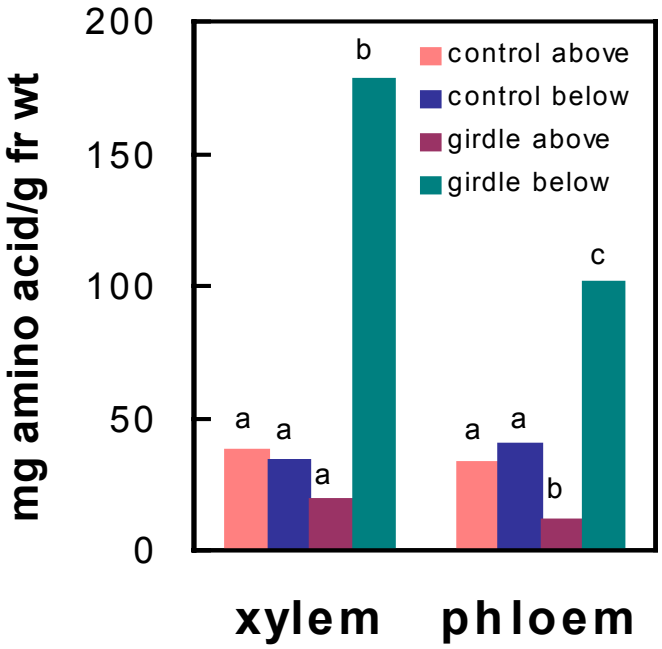
More evidence that N availability alters cell wall properties was obtained using pyrolysis molecular beam mass spectroscopy in collaboration with Dr. Mark Davis (NREL). The data below are taken from whole plants treated with varying levels of N availability.

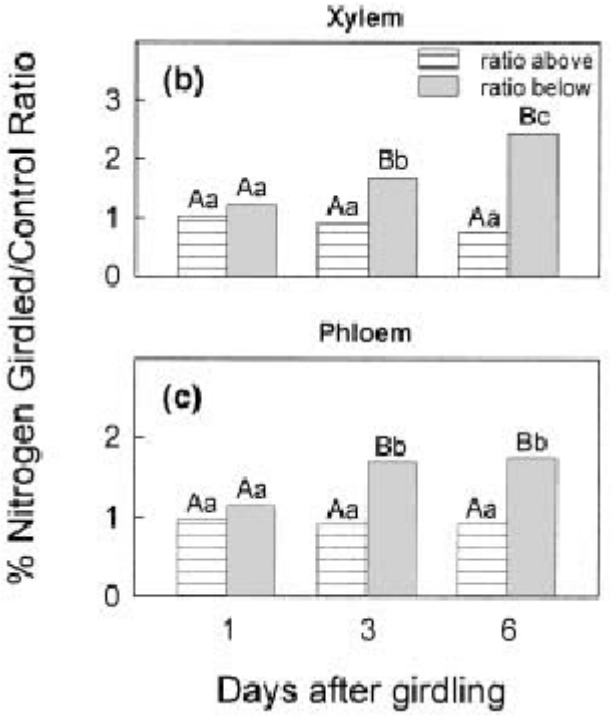


Pyrolysis molecular beam mass spectrometry (pyMBMS) of stem samples indicated that nitrogen content in the stems is negatively correlated with lignin content. Principal component analysis of spectra from nitrogen-treated plants. Peaks at m/z 84 and 129 are assigned to glutamine, while peaks at m/z 94, 110, 124, 137, 150, 154, 167, 180, 194, and 210 are associated with lignin. Peaks at m/z 110, 122, 124 are representative of low molecular weight phenols. n=4 independent replicates for the N fertilization experiments, 3 repetitions per sample.

In order to rapidly stimulate changes in N availability in xylem, we used phloem girdling. This approach effectively decouples the effects of plant development from our experiments (i.e., comparing rapidly growing plants to slowly growing plants). Girdling effectively and rapidly stimulated a large change in nitrogen availability in the xylem.

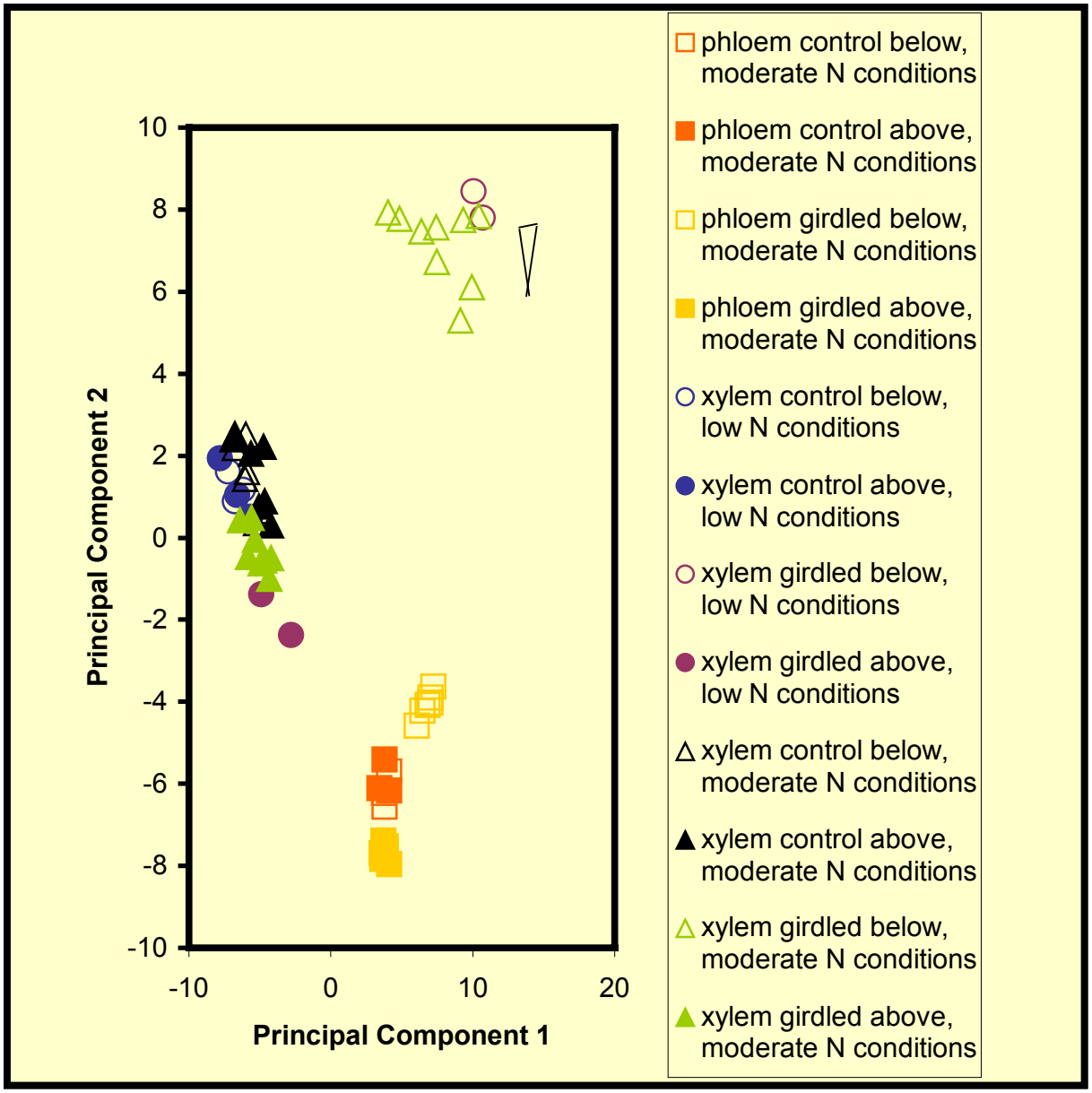






Girdling creates a nitrogen availability gradient within the stem. Upper Left, a photograph of a phloem-girdled stem with an illustration of how the transport amino acid glutamine, which is transported primarily via the phloem, is blocked and accumulates below the girdle while being depleted above the girdle. Upper Right, quantitation of this amino acid gradient. Lower Left, the time course of gradient development.

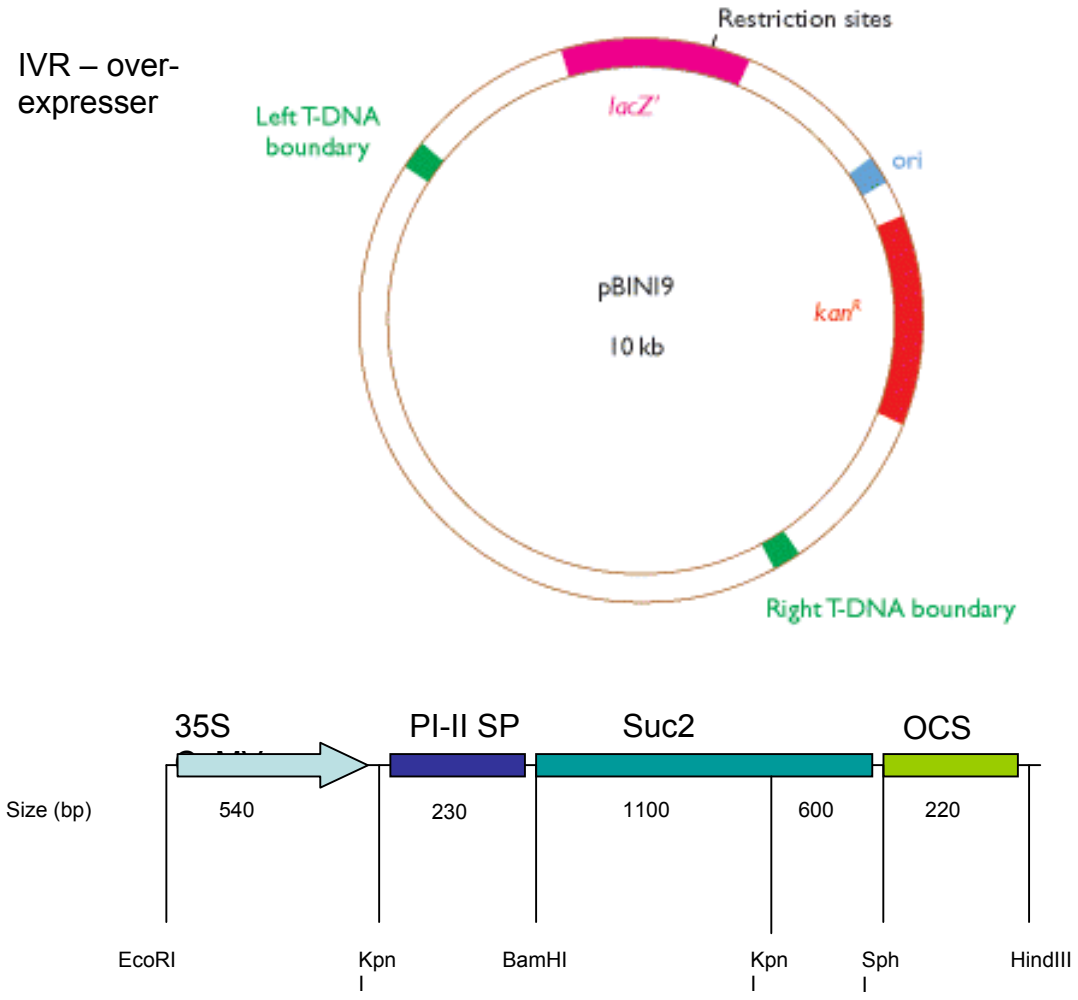
Notably, this gradient of N availability after girdling creates the same lignin content gradient that occurs in whole plant feeding experiments. Consequently, we were able to rapidly and reproducibly manipulate wood quality by altering N availability in whole plant feeding experiments (28 days), and by phloem girdling (< 6 days).



Pyrolysis molecular beam mass spectrometry (pyMBMS) of stem samples indicated that nitrogen content in the stems is negatively correlated with lignin content. Principal component analysis of spectra from nitrogen-treated (A, B) or stem-girdled plants (C, D). Peaks at m/z 84 and 129 are assigned to glutamine, while peaks at m/z 94, 110, 124, 137, 150, 154, 167, 180, 194, and 210 are associated with lignin. Peaks at m/z 110, 122, 124 are representative of low molecular weight phenols. n=4 independent replicates for the N fertilization experiments, n=3 independent replicates for the stem girdling experiments, 3 repetitions per sample.

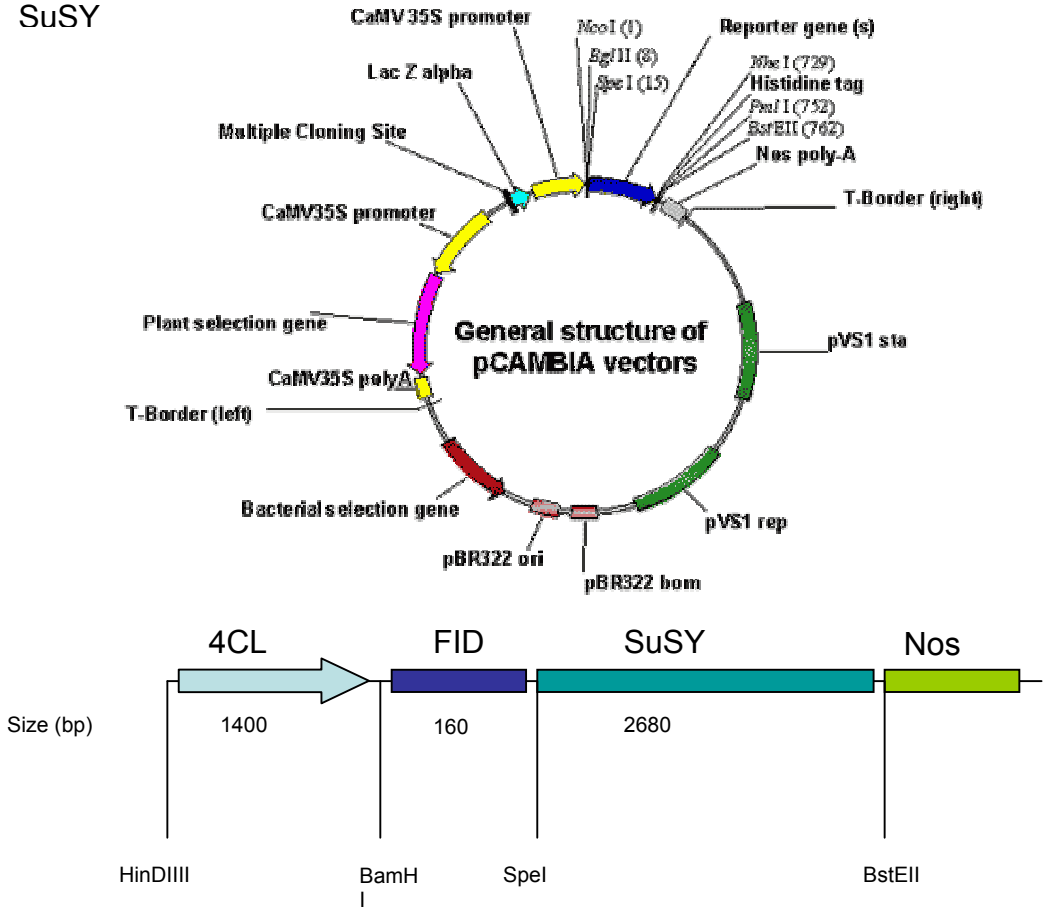
II.3.) To produce and analyze transgenic *Populus* trees with increased capacity to import C resources into wood.

The target genes for manipulation were cell wall Invertase, utilizing the yeast invertase SUC2 as the (non-inhibited) isoform to drive sink strength, and Sucrose Synthase from maize. Diagrams of the constructs follow:



Overexpression construct for cell wall invertase. The 35S promoter drives a transcript encoding a signal peptide from a potato proteinase inhibitor, the coding region of yeast SUC2, and the octopine synthase terminator.

4CL –Intron-SuSY



Overexpression construct for maize sucrose synthase. The 4CL (4-coumarate:coA ligase) from poplar was obtained from Carl Douglas and used to replace the 35S promoter from pCAMBIA. The FID is a "first intron derivative" from maize sucrose synthase that has been found to enhance gene expression.

The invertase plant lines generated by our collaborators were not confirmed to be transgenic by genomic PCR or expression-based analysis. The sucrose synthase transgenics harbored a population of tissue culture-borne fungal mites upon arrival and were lost due to contamination. We entered into a collaboration on a new project funded by a different program, where we are continuing this work. In this new project we designed new constructs containing a double 35S promoter and targeting yeast invertase to two different subcellular compartments: vacuole and cell wall. The new lines have been confirmed to be transgenic. Thus we will continue to collect phenotypic data beyond the scope of this project but will acknowledge support for this project in future publications.

Commercialization Opportunities:
Thus far no inventions have been made that would be good candidates for attracting licensees. Thus no formal commercialization agreements have been negotiated. As intellectual property is generated in this project, it is assessed for novelty and potential applicability in-house at UF, and we are alert to the possibilities. Potential application could derive from the usefulness of transgenic tree lines. However, there are potentially significant barriers to commercial deployment of transgenic trees from a social / political perspective that are not yet resolved.

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Davis JM. 2003. Physiological manipulation of nitrogen allocation in poplar trees. Department of Microbiology and Cell Sciences, University of Florida, April 21. Oral presentation.

Davis JM. 2003. Molecular determinants of carbon sink strength in wood. Agenda2020 review meeting, Asheville, NC, June 24. Oral presentation.

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Davis J. 2002. Trait-targeted genomics in forest trees. Department of Biological Sciences seminar series, University of Victoria, British Columbia. Oral presentation.

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Cooke JEK, Davis JM. 2001. Using a targeted genomics approach to examine nitrogen responses in poplars. Meeting of the IUFRO Working Group on Molecular Biology of Forest Trees, Stevenson, WA, July 22-27. Oral presentation.

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Davis JM. 2001. Molecular analysis and regulation of growth in poplar trees. OIT Agriculture Biobased Products Portfolio Review, Washington, D.C., February 22. Oral presentation.

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Cooke JEK, Brown KA, Orozco E, Balangue CA, Davis JM. 2000. Gene expression associated with shifts in carbon and nitrogen allocation in Populus. Sixth International Congress of Plant Molecular Biology, Quebec City, June 18-24. Poster Presentation.

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